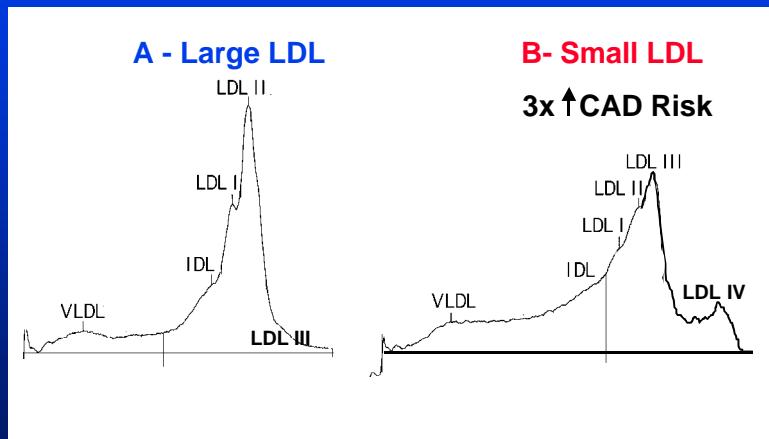
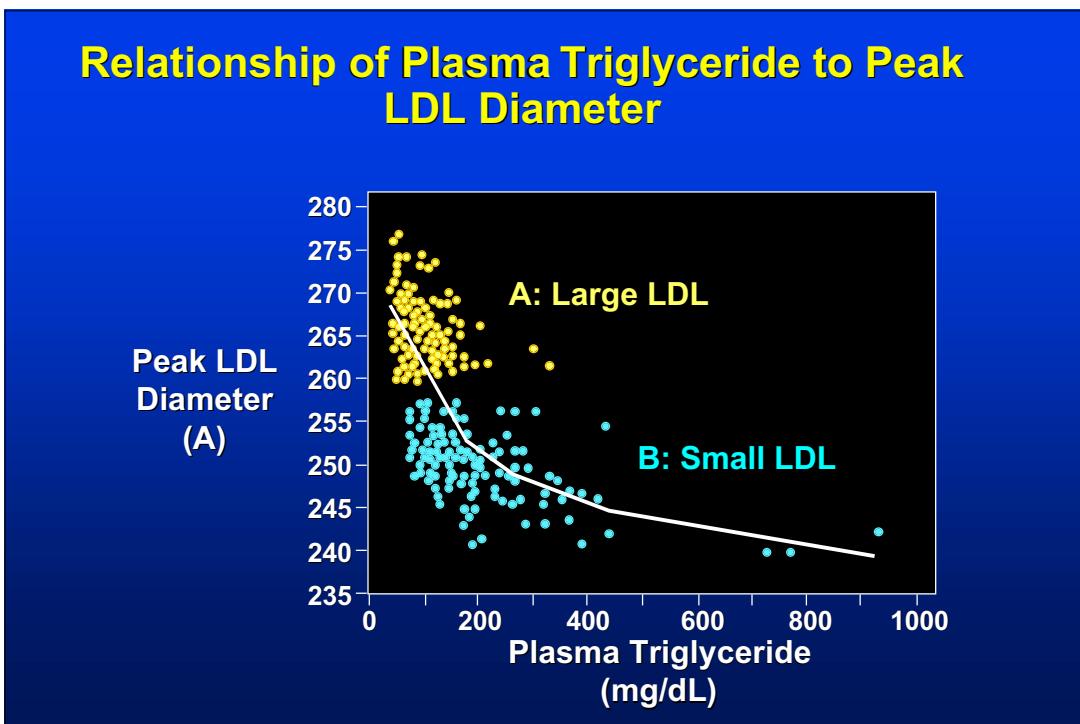
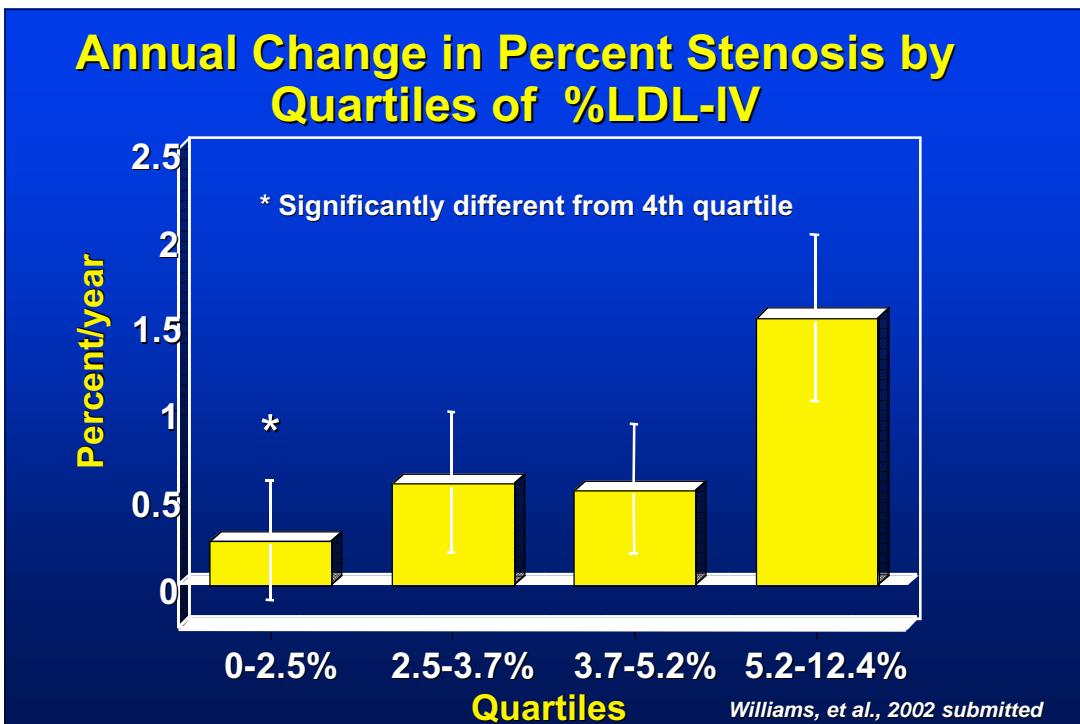


Genetic and Genomic Tools to Decipher Atherogenic Lipoprotein Metabolism

Ronald M. Krauss
Lawrence Berkeley National Laboratory
University of California
Berkeley, California

Multiple LDL Subclasses Distinguished by Size Using Gradient Gel Electrophoresis

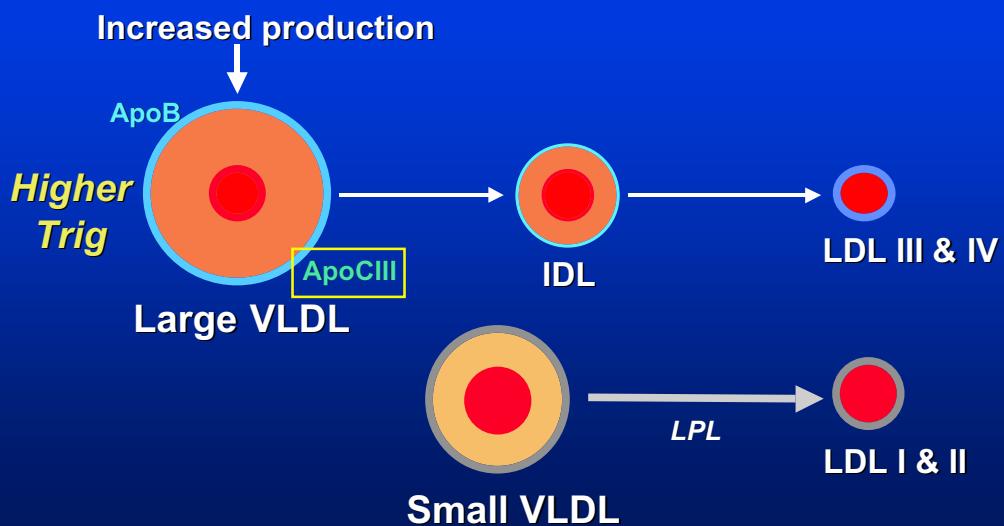




What Are Determinants of LDL Subclass Profiles?

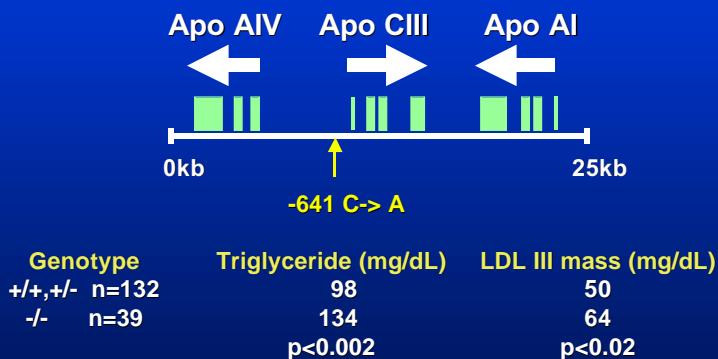
- Genetic influences underlying bimodal LDL size distribution have been shown by segregation and linkage studies
- Metabolic relationships are suggested by association with plasma triglyceride
- Can genetic studies yield information that could elucidate determinants of LDL subclasses?

Atherogenic Lipoproteins

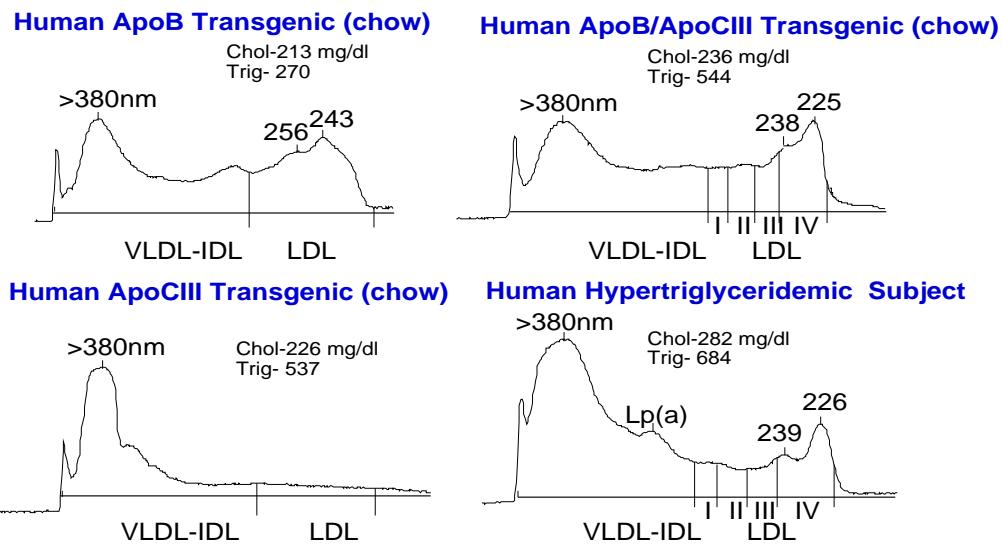


Krauss AHJ 1987; Packard and Shepherd, ATVB 1999.

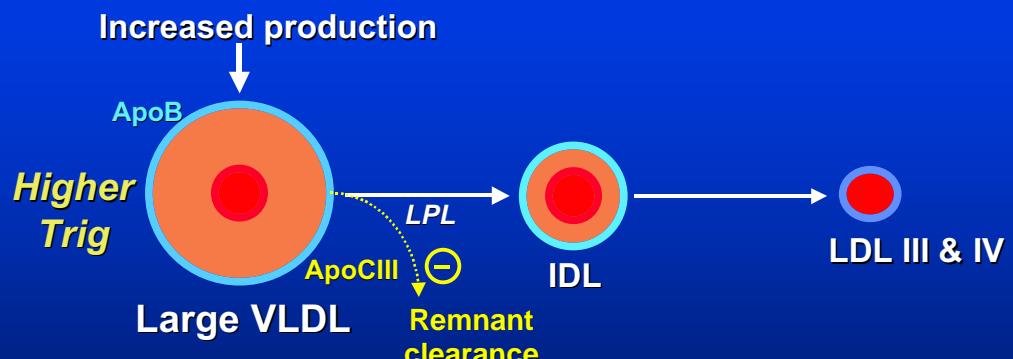
Association of Triglyceride and Small LDL with ApoCIII Promoter Polymorphism



Increased Small LDL in Human ApoB/ApoCIII Transgenic Mice and Human Hypertriglyceridemia

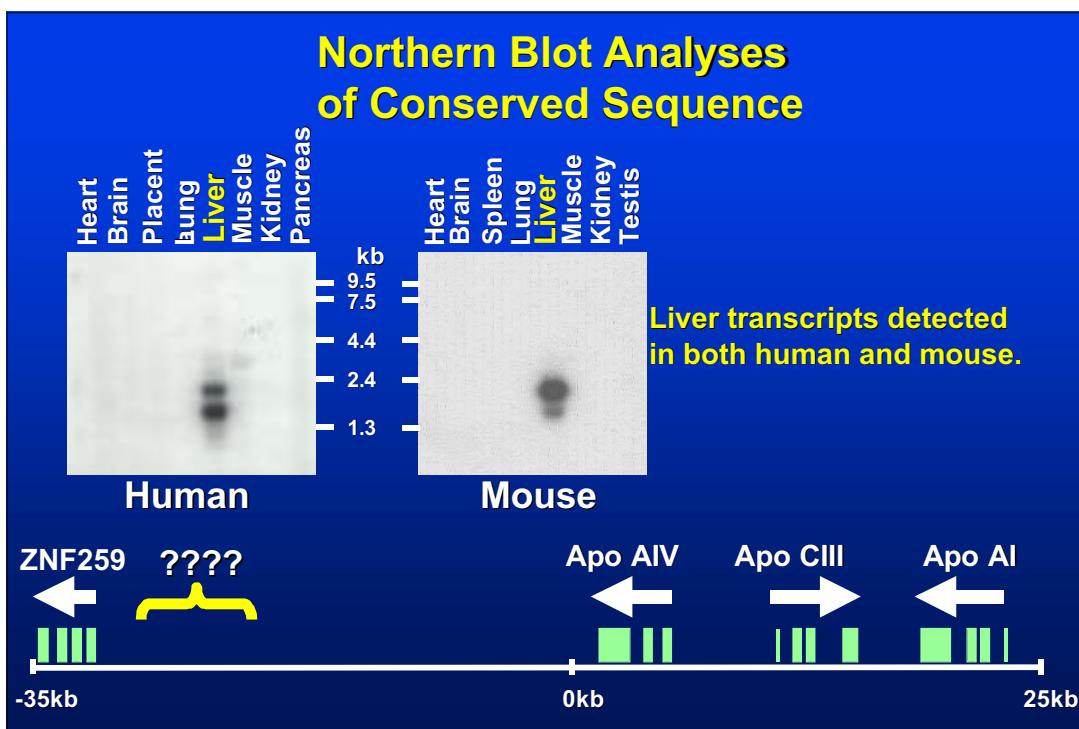
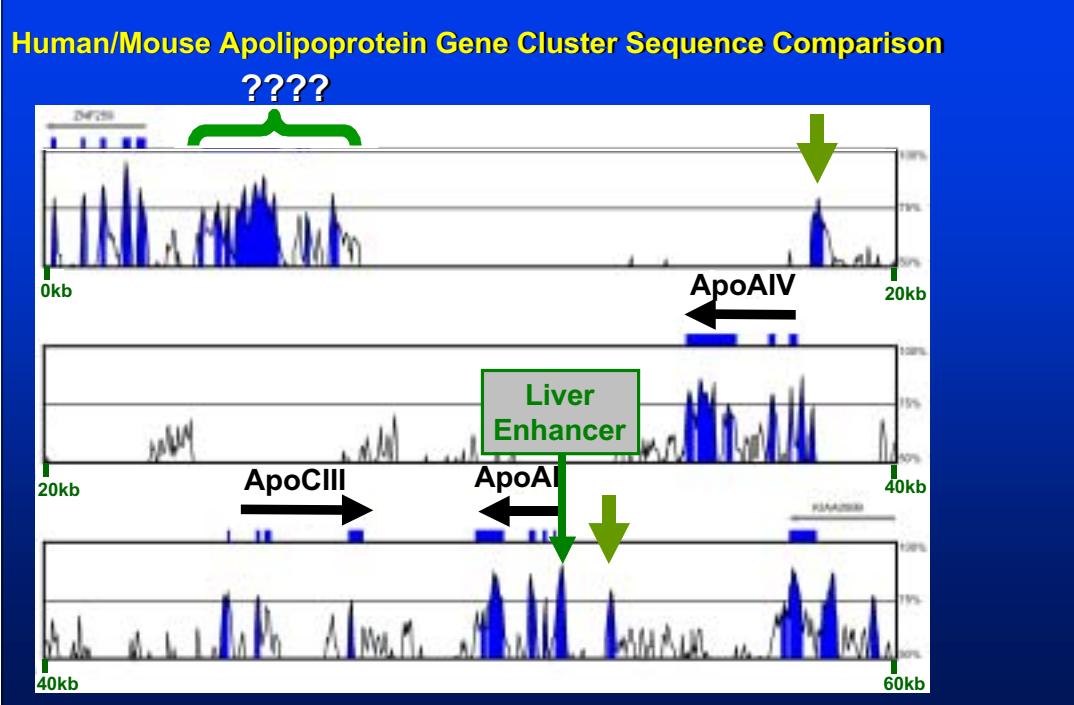


Atherogenic Lipoproteins



Chromosome 11q Apoprotein Gene Cluster





Predicted protein has homology to ApoAIV

**predicted protein
human apoAIV**

```
--MAAVLIVALALIS---AFSAIQARKGFWDYFSQTSQG-DKGRVEQDQH  
MFLKAIVLILAPVAAGARAEVSAQAVATVMWDYFSOLSNNAKEAVBHQ  
  
QQKAREP-ATLKDSLEQDLNNMNKFLEKIRPLSGSEAPRLPODPVGMR  
KSE-TQQLNALFQDIEGEVNTYAGDLQRKLVEFATELHERIAKDSERKKE  
  
QIQQEELEEVKARLOPYMAEAHELGWNIEGCROQIKPYTMIDMEQVALRV  
EIGKELEELRARIILPHANEVSQKICDNIREQORIEPVADQRLRQVNTQA  
  
QEIQEQLRVVGEDTKAOQLGGVDEAWALIQQG---IQSRRVHHTGREKEL  
EQIRROLDPEAORMERVIRENAIDLQASLRPHADELKAKIDQNVEELKGR  
  
FHBYA-SLVSGIGREVOELRSVAHAPASPARSRCQVLSRKITLKK  
LTBYA-EFKVKIDQIVELRRSLAYAODIEKINHQEGLIFQVKKNAE  
  
ALHARTQNLDOIRELSRAFAGT----CTEECAGPDPMISEEVPRD  
ELHARTSASABEPLRQLAPLAEDVRGNLKGNTIECLQKSLABLGHDQV  
  
QAFRQDTYLQIAAFTRAIDQETEEQQLAPPPPGHSFAPEFQQTDSGK  
EEFRRRVEPYGENPKAVVQOMEOIROLGPCHAGDVECHLSFLEKDLRDK  
  
VLSKLQARLDLWEDIITHSIHQGHSHLGDP-----  
VNSFFSTFKCESQDKTLSPLEQQQEQQEQQQEQVQMLAPLES
```

**Identity: 26%
Similarity: 45%**



Predicted protein has homology to ApoAIV

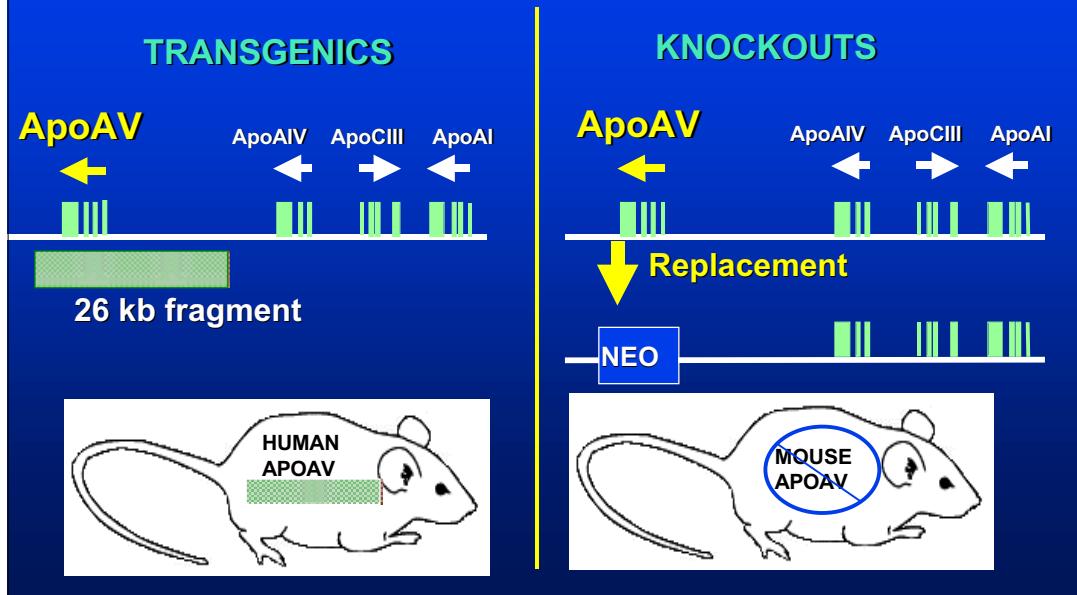
**predicted protein
human apoAIV**

```
--MAAVLIVALALIS---AFSAIQARKGFWDYFSQTSQG-DKGRVEQDQH  
MFLKAIVLILAPVAAGARAEVSAQAVATVMWDYFSOLSNNAKEAVBHQ  
  
QQKAREP-ATLKDSLEQDLNNMNKFLEKIRPLSGSEAPRLPODPVGMR  
KSE-TQQLNALFQDIEGEVNTYAGDLQRKLVEFATELHERIAKDSERKKE  
  
QIQQEELEEVKARLOPYMAEAHELGWNIEGCROQIKPYTMIDMEQVALRV  
EIGKELEELRARIILPHANEVSQKICDNIREQORIEPVADQRLRQVNTQA  
  
QEIQEQLRVVGEDTKAOQLGGVDEAWALIQQG---IQSRRVHHTGREKEL  
EQIRROLDPEAORMERVIRENAIDLQASLRPHADELKAKIDQNVEELKGR  
  
FHBYA-SLVSGIGREVOELRSVAHAPASPARSRCQVLSRKITLKK  
LTBYA-EFKVKIDQIVELRRSLAYAODIEKINHQEGLIFQVKKNAE  
  
ALHARTQNLDOIRELSRAFAGT----CTEECAGPDPMISEEVPRD  
ELHARTSASABEPLRQLAPLAEDVRGNLKGNTIECLQKSLABLGHDQV  
  
QAFRQDTYLQIAAFTRAIDQETEEQQLAPPPPGHSFAPEFQQTDSGK  
EEFRRRVEPYGENPKAVVQOMEOIROLGPCHAGDVECHLSFLEKDLRDK  
  
VLSKLQARLDLWEDIITHSIHQGHSHLGDP-----  
VNSFFSTFKCESQDKTLSPLEQQQEQQEQQQEQVQMLAPLES
```

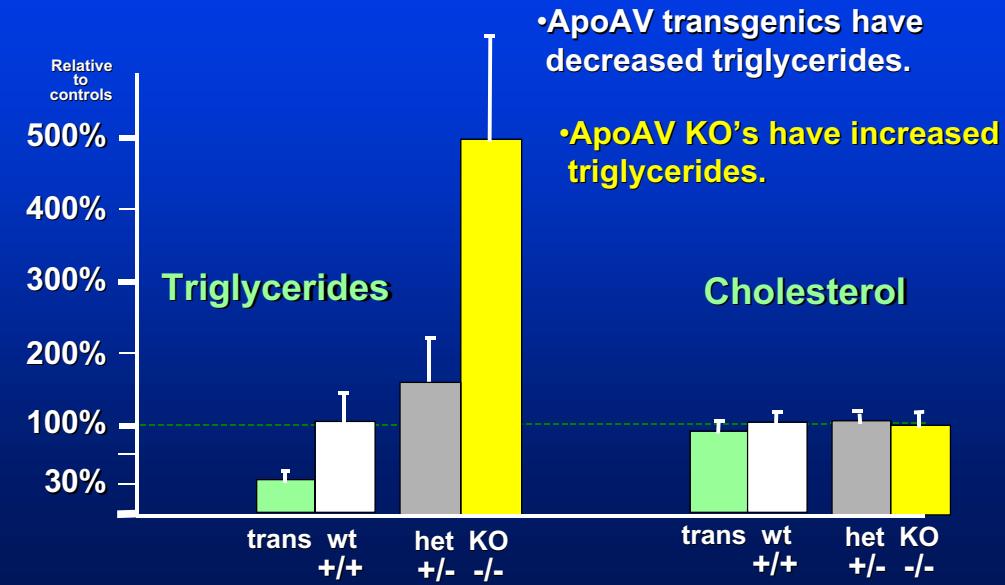
**Identity: 26%
Similarity: 45%**

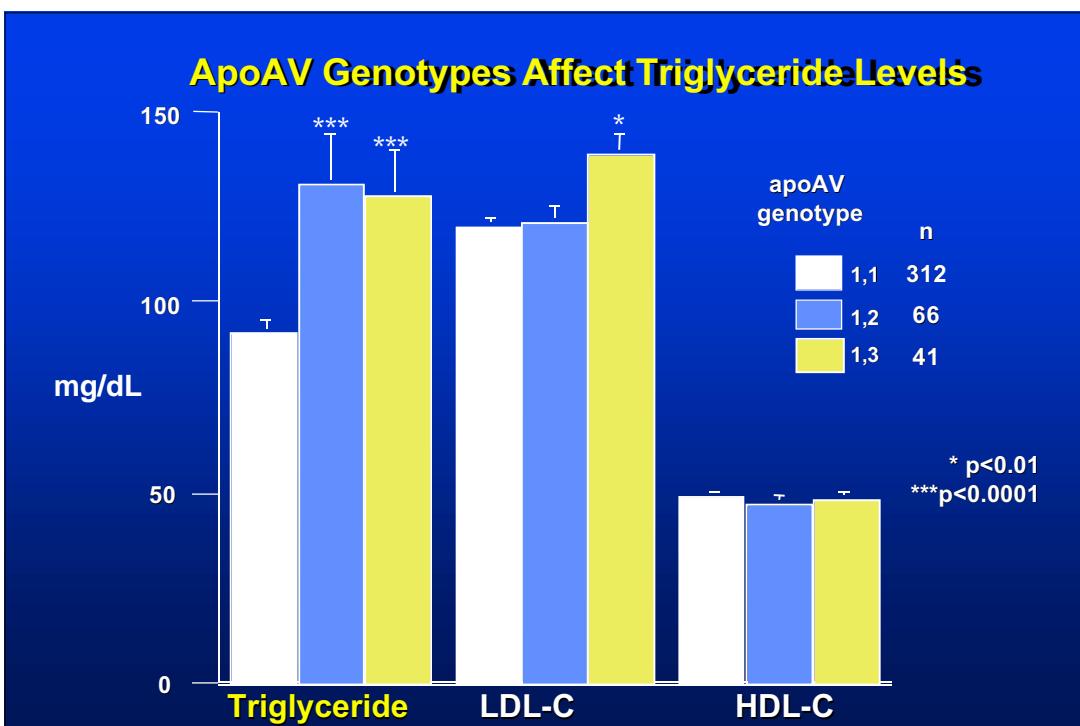
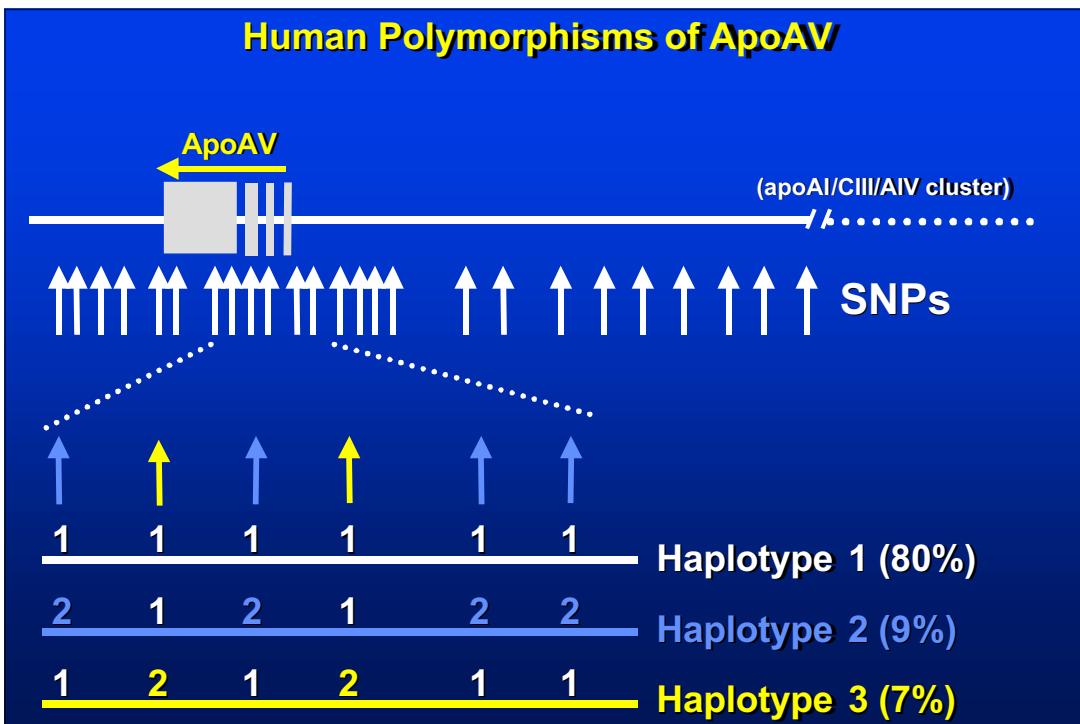


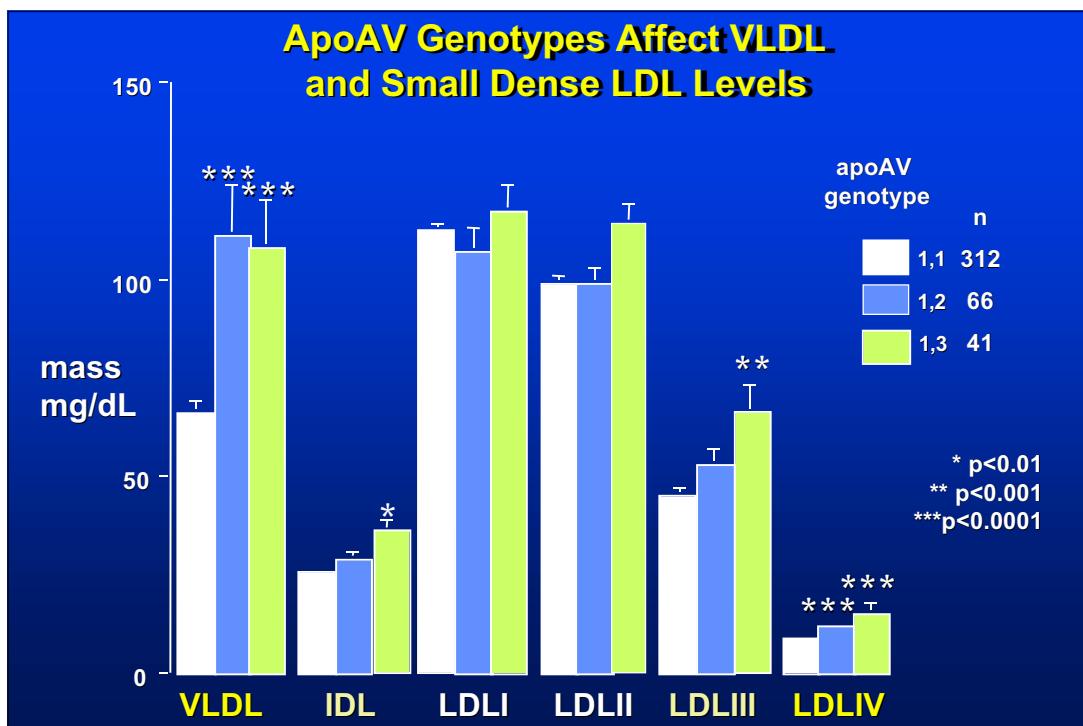
ApoAV Mouse Studies



ApoAV Transgenic and Knockout Plasma Levels



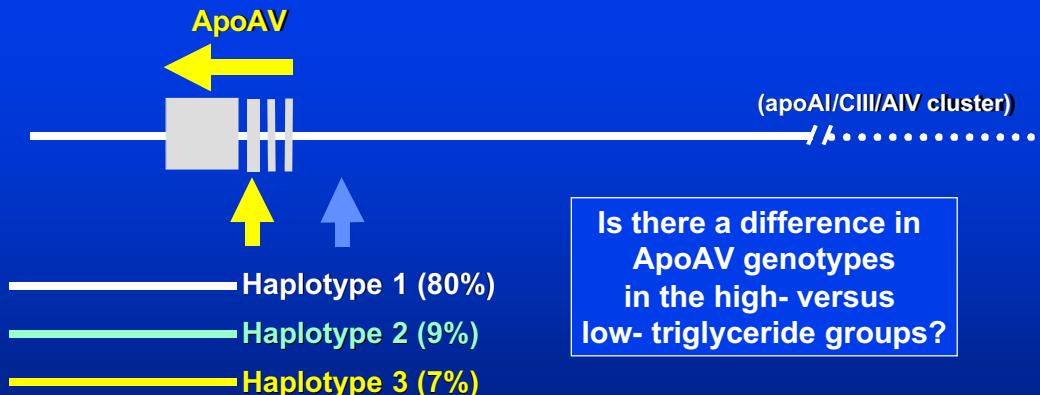




Association Studies.....

Is this finding reproducible????

Association study 2: ApoAV polymorphisms and plasma lipids



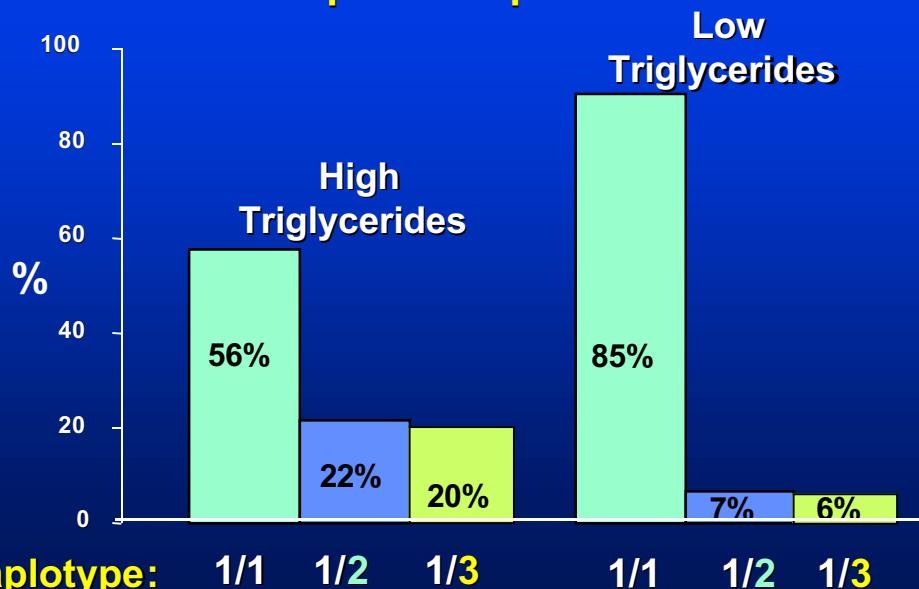
Is there a difference in
ApoAV genotypes
in the high- versus
low- triglyceride groups?

Genotyped 460 individuals stratified based on trig levels.

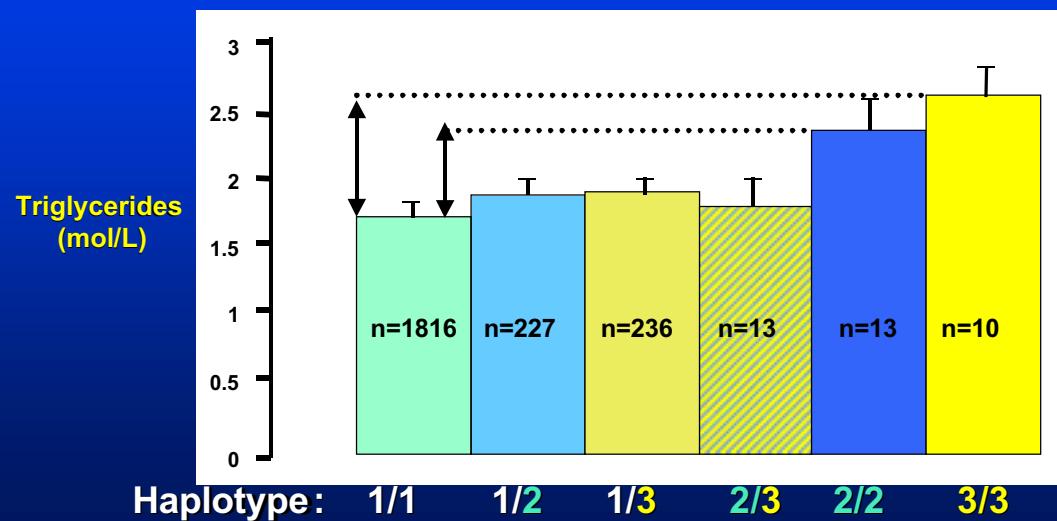
High Triglycerides: Avg 340 mg/dl

Low Triglycerides: Avg 50 mg/dl

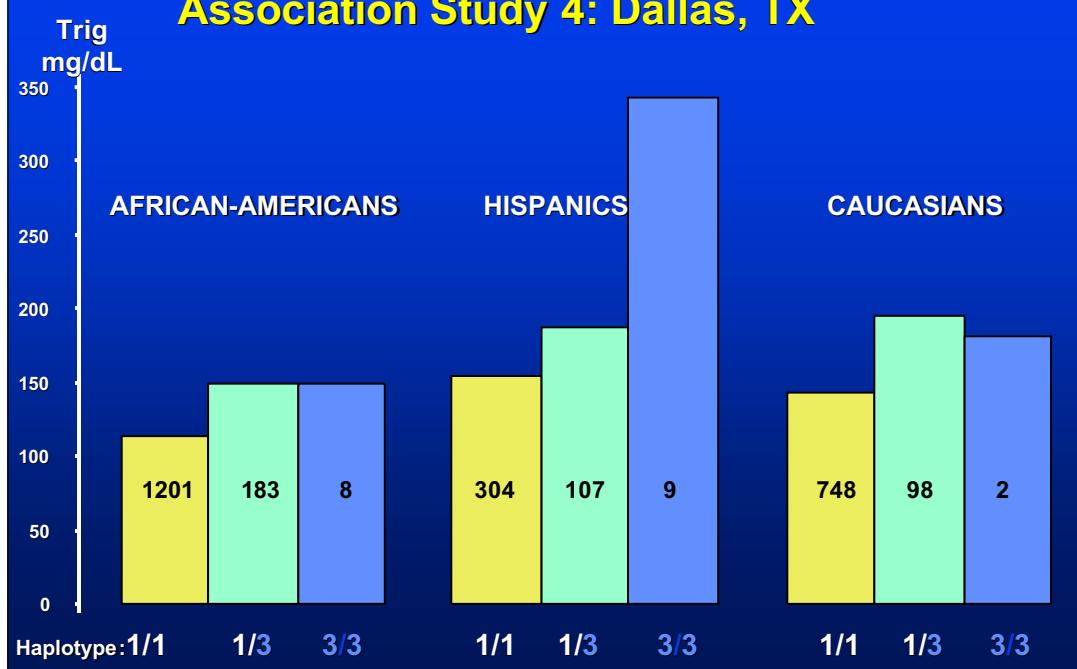
Association study 2: ApoAV polymorphisms and plasma lipids



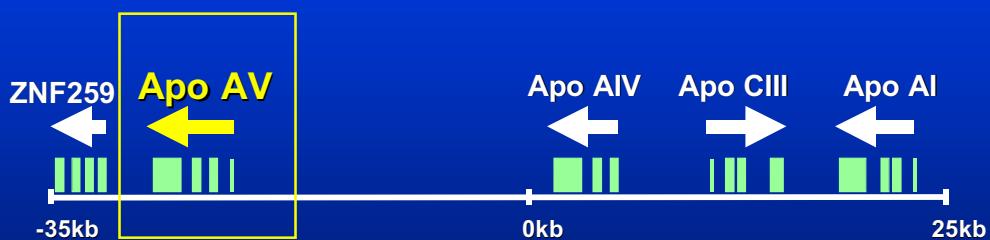
Association Study 3: Analysis in 2200 English Males



Association Study 4: Dallas, TX

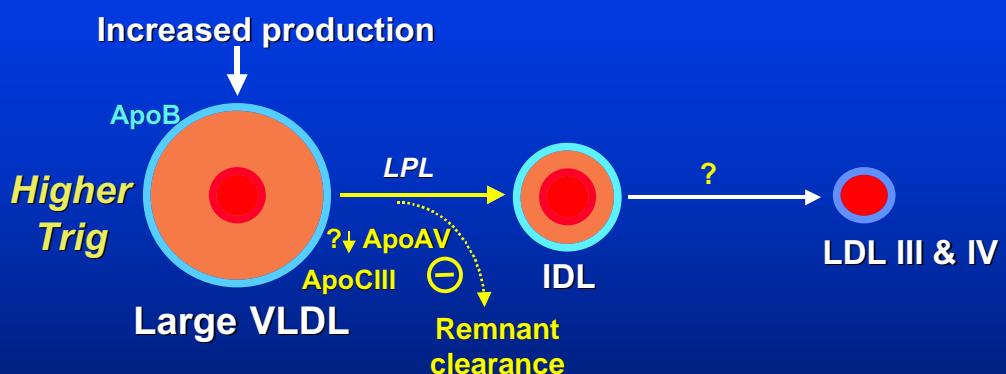


Updated Apolipoprotein Gene Cluster Human Chromosome 11q23

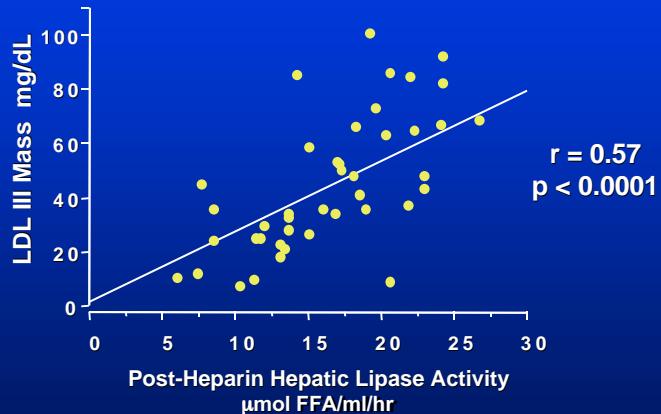


Pennacchio et al. *Science* 294:173, 2001

Atherogenic Lipoproteins



Hepatic Lipase Activity is Correlated with LDL III Mass in Healthy Subjects



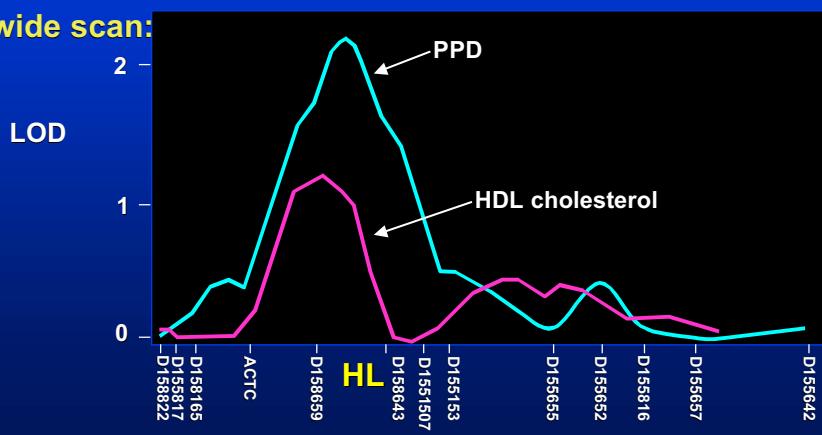
Campos et al., JLR 36:462, 1995

Linkage of LDL Size (n=498) and HDL-C (n=720) to the Hepatic Lipase Gene Locus in FCHL

1) Quantitative sibpair linkage analysis:

D15S643: LDL diam $p < 0.019$, HDL-C, $p < 0.003$
D15S148: LDL diam $p < 0.008$, HDL-C, $p = 0.1$

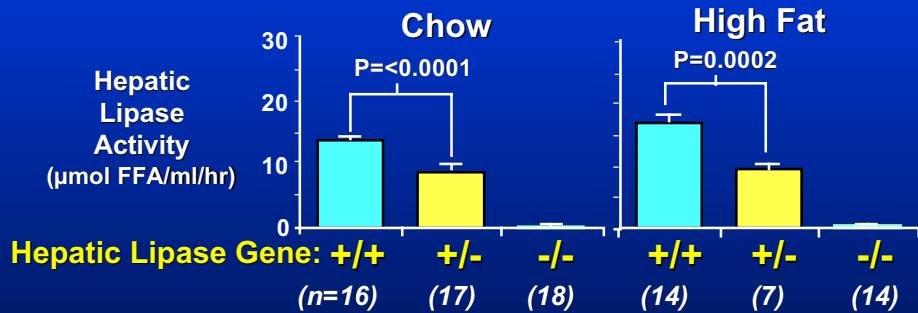
2) Genome-wide scan:



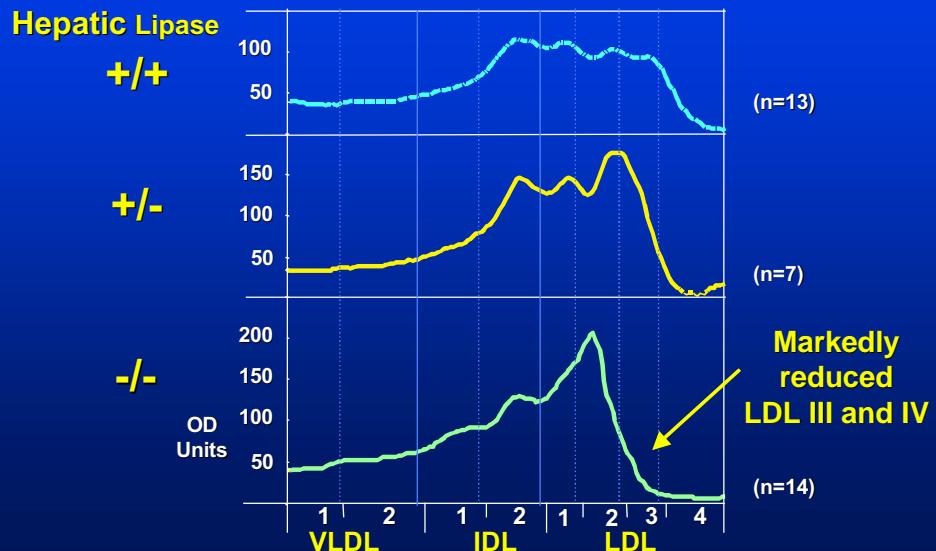
Allayee H et al. J Lipid Res. 2000;41:245-252.

Hepatic Lipase Deficient Human-ApoB Transgenic Mice

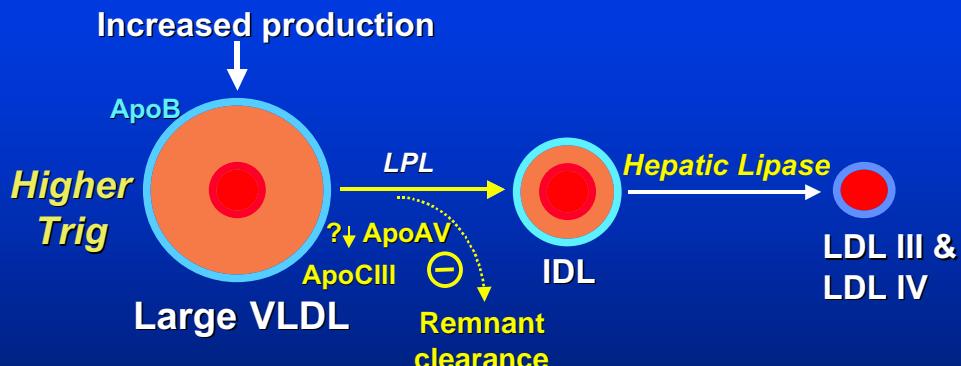
- Bred from Human-apoB (Callow et al., 1994) and HL knock out (Homanics et al., 1995) into FVB background



Production of Small LDL Particles in ApoB Transgenic Mice is Absolutely Dependent on Hepatic Lipase Activity



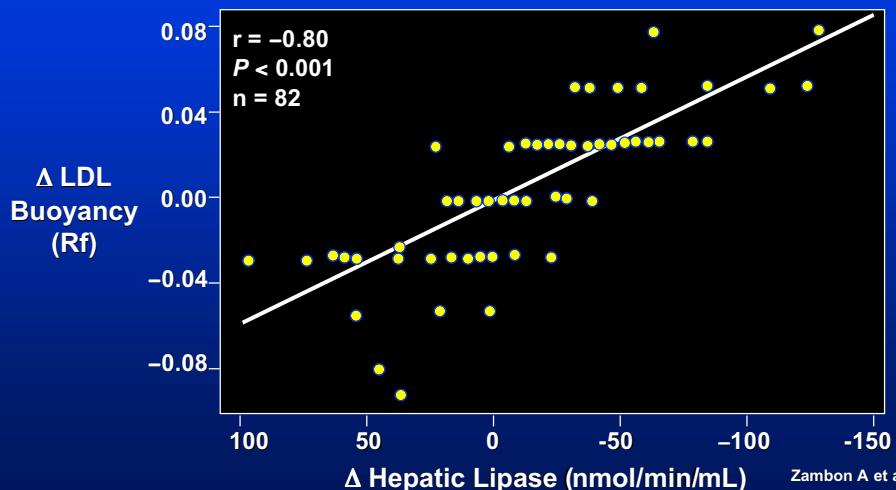
Key Influences on Formation of Small LDL



What are the clinical implications of this pathway?

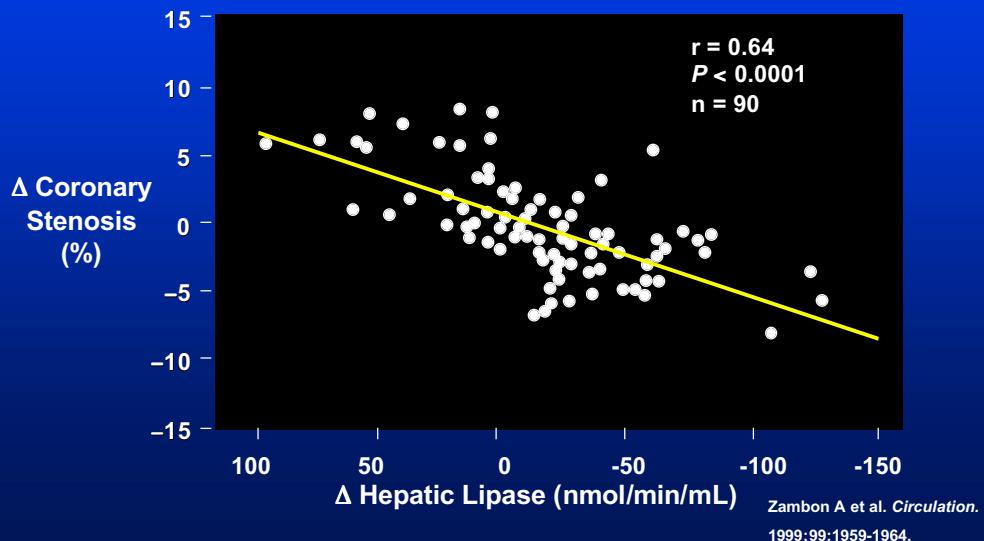
FATS

Δ Hepatic Lipase and Δ LDL Buoyancy (~Size)



Zambon A et al. *Circulation*.
1999;99:1959-1964.

FATS Δ Hepatic Lipase and Δ Coronary Stenosis



Conclusions: Genes That Influence Small Dense LDL

- Common haplotypes of ApoAV, a newly discovered gene that affects plasma triglyceride levels, also influence levels of the smallest, most atherogenic LDL particles
- A critical role for hepatic lipase in production of these small dense LDL is demonstrated in hepatic lipase knockout mice
- Reduction in hepatic lipase activity in humans may add to benefit of hypolipidemic therapy by reducing production of small dense LDL

Acknowledgments

**Pat Blanche
Laura Holl
Joseph Orr**

**Apo AV Studies:
Len Pennacchio
Eddy Rubin Lab
Michael Olivier
Jonathan Cohen**